REMARKS/ARGUMENTS

Claims 95—98 are active in this case.

Claim 95 has been amended to incorporate previously submitted, but now cancelled, Claim 108 defining the cultured human cells as T-cells. The fact that the cells so cultured have enhanced cytokine secretion is disclosed on page 11, lines 6-7 and pages 23-26.

The specification is amended to insert a substitute Title.

No new matter is added

The claims of this application are directed to methods of obtaining human T cells with enhanced replicative function. Those cells are cultured under certain conditions as defined in the claims. As discussed in the specification, an advantage of the present invention is the discovery that culturing cells, including T-cells, one can obtain a population of cells with enhanced replicative function making these cells particularly useful for therapeutic applications.

On page 5 of the Action an obviousness rejection citing U.S. 5,994,126 is outlined but then further refers to the US '556 patent. It is believed that this is the previous rejection citing the '126 patent and the inclusion of US '556 was simply an inadvertent error. Therefore, the rejection under 35 USC 103(a) in view of the US '126 patent is discussed further below.

The rejection is not tenable because this cited patent does not describe or otherwise suggest culturing T-cells as claimed. Further, the culturing of T-cells as set forth in the claims and demonstrated in the examples was not reasonably predicted from what is actually taught in the US '126 patent and the knowledge in this field.

The results for T-cells as shown in the specification are unexpected and novel notwithstanding what US '126 describes pertaining to dendritic cells. For example, T-cell concentrations for conventional processes in tissue culture flasks or gas permeable culture

bags are maintained optimally at 10^5 to 10^6 per ml or less based on common knowledge in the field of cell culture. Conventional wisdom suggests that when T-cells achieve a maximum cell concentration of 1-3 million cells per ml, the cultures must be diluted and split immediately into multiple cultures such that density is restored to 5 x 10^5 cells/ml or less (i.e. "hemi-depletion" to maintain low cell density by adding medium and/or splitting individual cultures into multiple cultures). Typically, this process is repeated several times (generally every 2-7 days during the course of standard T-cell culture (See Riddell and Greenberg, 1990 *J Immunol Methods 128*:189-201, attached, for one representative example of this approach for T-cells).

These standard processes are costly, labor intensive and consist of multiple manual open-process steps which are difficult or impossible to implement for multiple samples under stringent regulatory requirements. These constraints are a major limitation to the widespread evaluation and implementation of adoptive T-cell based immunotherapies in large multi-center Phase II and Phase III clinical trials, potentially leading to widespread delivery and commercialization of therapy.

In contrast, as described in the Examples of the application (see discussion starting at page 23) T-cells can be inoculated at low density (10^3-10^5 /ml) and grown to high density (10-40 x 10^6 cells/ml) in a single continuous process without splitting or sub-culture using frequent medium exchange methodology as set forth in the claims. In addition, medium exchange can be ramped based on lactate production so as to maintain a lactate tolerance level of 0.5 – 1.0 mg/ml as determined by sampling and measurement in the waste medium.

Obtaining T-cells with enhanced proliferative potential and cytokine secretion under these continuous culture conditions is entirely unexpected and counter-intuitive to those skilled in the art as indicated above (i.e. conventional wisdom emphasizes splitting cultures to maintain low density).

"The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results.".*KSR v. Teleflex*, 550 U.S. ___, 127 S. Ct. 1727 (2007). Here the applicants have made it quite clear that the methodology claimed was contrary to conventional wisdom for T-cells and what was obtained, in terms of enhanced proliferation and cytokine release, was entirely unexpected from the teachings of US '126 and that conventional wisdom.

The Applicants have observed unexpectedly enhanced activity as measured in such bioassays including cytokine release and proliferation compared to conventional low density hemi-depletion cultures.

Furthermore, T-cells derived in the examples proliferate more vigorously (after culture to densities exceeding 10-40 x 10^6 per ml in a single bioreactor cassette) and also release cytokines at higher levels than T-cells cultured in parallel under conventional low density (<1-3 x 10^6 T-cells per ml) using hemi-depletion methods. Thus, continuous culture under perfusion conditions does not have deleterious effects on cell division and may prevent or minimize immunological senescence. This unexpected result when considering conventional culture (as highlighted above) forms the basis of the invention consisting of continuous low to high density T-cell expansion without subculture under medium perfusion conditions. This T-cell culture strategy and potential benefits on proliferative potential or cytokine release would not be obvious to one skilled in the art.

Accordingly, withdrawal of this rejection is requested.

The rejection under 35 USC 112, first paragraph is respectfully traversed. From the rejection outlined on pages 3-4, a main issue would appear to be the phenotypes of the cells after culturing, i.e., the previous phrase "enhanced biological function" notwithstanding the

further limitation that this function comprised enhanced replicative function. (see also the discussion of the term "comprising" in the claims as part of the basis for maintaining the rejection (page 3 of the Official Action).

It should be noted that the claims have been amended to remove "enhanced biological function" and have defined that the cultured human T-cells have enhanced replicative function and cytokine release as discussed in the specification and shown in the examples.

Therefore, on this basis alone, it is believed that this rejection is no longer applicable to the claims as presented herein.

Addressing the points in the rejection:

On page of the Action, it states that "cultures....will reach a maximum density, undergo contact inhibition and cease division." Furthermore, "failing to passage cells at appropriate times before they become overgrown can cause a range of deleterious effects in the cells that may result in changes in the characteristics of the culture which may be permanent and alter the response of cells in bioassays or other applications."

However, as discussed above in the context of the obviousness rejection, the Applicants have observed unexpectedly enhanced activity as measured in such bioassays including cytokine release and proliferation compared to conventional low density hemi-depletion cultures.

Furthermore, as discussed above continuous culture under perfusion conditions does not have deleterious effects on cell division and may prevent or minimize immunological senescence. This unexpected result when considering conventional culture (as highlighted above) forms the basis of the invention consisting of continuous low to high density T-cell expansion without subculture under medium perfusion conditions.

While *in vitro* assays may or may not reliably predict therapeutic efficacy in patients, the claims are directed to a method of culturing cells not therapy per se. However, note that

cytokine release is currently used as a potency assay for therapeutic T-cells (see attached, Dudley et al (2002).*Science 298*:850-854; and (2005) *J. Clin. Oncol.* 23(10): 2346-57).

The results indicating increased post-harvest proliferation and cytokine release may reflect the high metabolic activity of T-cells harvested from perfusion cultures or other biological effects of perfusion in combination with the unique microenvironment and cellular interactions produced in high density cultures. In particular, clinical activity has been shown to correlate positively with the ability of T-cells (including Tumor infiltrating lymphocytes or "TILs") to produce GM-CSF (Cole et al., 1994 *Cancer Immunol Immunother 38*:299-303; Schwartzentruber et al., 1994 *J Clin Oncol 12*:1475-1483; Ridolfi et al., 2003 *J Immunother 26*:156-162, all attached). Note that GM-CSF is produced at high levels by T-cells described in the Examples of the specification.

Also note that the capacity for proliferation may also provide a key function for T-cell therapeutic efficacy. For example, long term differentiation and expansion of T-cells is associated with telomere shortening (Rufer et al., 1998 *Nat.Biotechnol.* 16:743-747; Reed et al., 2004 *J.Exp.Med.* 199:1433-1443) and proliferative "exhaustion" or senescence. Shortening of telomeres is associated with loss of T-cell function including proliferation (Blackburn, 2001 *Cell* 106:661-673; de Lange, 2002 *Oncogene* 21:532-540). Furthermore, it has been reported (Zhou et al. (2005) *J.Immunol.* 175:7046-7052) that T-cells demonstrating the greatest therapeutic efficacy against cancer had the greatest telomere lengths, highest proliferative potential and longest persistence *in vivo* in humans. In contrast, telomere shortening was associated with loss of proliferative potential, poor persistence *in vivo* and lack of objective clinical responses in human patients as measured by tumor shrinkage and patients' survival.

Therefore, high proliferative potential and cytokine release at the time of harvest as shown in the examples and methods of the present application support their potential for use in therapeutic applications.

Withdrawal of the rejection is requested.

Applicants also request a notice of allowance confirming the allowability of all pending claims.

Should the Examiner deem that any further action is necessary to place this application in even better form for allowance, he is encouraged to contact Applicants' undersigned representative at the below listed telephone number.

Respectfully submitted,

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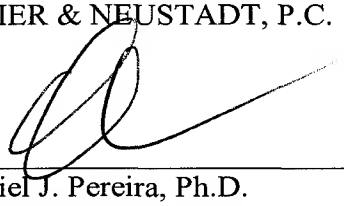
MAIER & NEUSTADT, P.C.



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